expectation of success, *i.e.*, that the invention would be operable. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. § 2142. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicant's disclosure (M.P.E.P. citing in favor, *In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

Applicants submit that the '134 patent and Yershov both suffer from the same defects and, accordingly, whether taken singly or in combination, do not disclose or teach the methods of the present invention. First, both references teach away from the use of a complete set of n-mers in their sequencing methods -- the goal of both references is to use a set of probes that is smaller than the complete set of n-mers. Second, both references state that their goal is to sequence a nucleic acid.

Claim 1 is directed to a method of determining the presence of a mutation in a target polynucleotide, which involves separately hybridizing target and reference polynucleotides to identical polynucleotide probe arrays to generate a target and reference hybridization patterns and determining the presence of a mutation in the target polynucleotide by comparing the reference and target hybridization patterns without sequencing the target polynucleotide. Claim 1 also states that each probe in the identical polynucleotide probe arrays has a double stranded region and a single-stranded n-mer overhang region such that the overhangs in each array constitute a complete set of n-mers.

Claim 12 is directed to a method of determining whether two or more target polynucleotides are identical without sequencing the target polynucleotides, by separately hybridizing a first and a second target polynucleotide to generate a first and second hybridization pattern and comparing the first and second hybridization patterns. Claim 12 also states that each probe in the identical polynucleotide probe arrays has a double stranded region and a single-stranded n-mer overhang region such that the overhangs in each array constitute a complete set of n-mers.

With regard to the use of a complete set of n-mers, Yershov teaches that their methods can make microchips with about 20,000 - 30,000 probe types, but recommends using the "contiguous stacking hybridization" improvement with 65,536 octamer probes that only

"simulates" the use of a complete set of 67,108,864 13-mers. Yershov at 4913, col. 1, last paragraph to col. 2, second paragraph. Hence, Yershov teaches that the "technical problems" of the sequencing by hybridization to oligonucleotide microchips (SHOM) technique (page 4913, col. 1, last paragraph) should be solved by not using a complete set of n-mers.

Moreover, the microchips allegedly produced by Yershov only "may contain" 20,000 - 30,000 probe types.

> Here we report an advance in the developments of SHOM technology and SHOM application for sequence analysis and diagnostics. A robot has been constructed for manufacturing microchips. Oligonucleotides are applied by the robot and immobilized into microchip elements fixed on a glass plate. A chip that is 1 × 1 cm may contain 20,000 - 30,000 40 × 40 μm elements.

Yershov at 4913, col. 1, last paragraph. But Yershov has not actually made a microchip with even 20,000 - 30,000 probe types. The Examiner has cited Yershov Fig. 3 as allegedly teaching certain aspects of the present invention. However, as indicated by the figure legend, Fig. 3 involves the use of only six probe types. Accordingly, one of skill in the art would understand from Yershov that making a microchip with a complete set of n-mers is not feasible and that a modified microchip or procedure using less than a complete set of n-mers must be used. Applicant submits that the teachings of Yershov would only serve to discourage one of skill in the art from using the claimed complete set of n-mers.

Combining the '134 patent with the teachings of Yershov does not cure the defects inherent in Yershov. The '134 patent teaches as follows.

> [I]f the random portion consisted of a four nucleotide sequence of adenine, guanine, thymine, and cytosine, the total number of possible combinations would be 4^4 or 256 different nucleic acid probes. If the number of nucleotides in the random sequence was five, the number of different probes within the set would be 4^5 or 1,024. This becomes a very large number indeed when considering sequences of 20 nucleotides or more.
>
> However, to determine the complete sequence of a nucleic acid target, the set of probes need not contain every possible combination of nucleotides of the random sequence to be encompassed by the method of this invention. . . For a nucleic acid sequence of length k, there are $4(2^k-1)$ instead of 4^k probes. Where $k = 8$, a set of probes would consist of only

> 1020 different members instead of the entire set of 65,536. The savings in time and expense would be considerable.

Col. 6, lines 6-17. Accordingly, the '134 patent advocates using less than a complete set of n-mers. The '134 patent goes on to teach that customized probes can be made which are "structurally useful for identifying and binding to only those sequences which are homologous to the overhangs." Col. 10, lines 24-25, *see also*, col. 10, lines 11- 67. Such teachings would discourage one of skill in the art from using a complete set of n-mers. Accordingly, the combination of Yershov and the '134 patent would guide one of skill in the art away from developing probe arrays having a complete set of n-mers as claimed by the present application.

With regard to nucleic acid sequencing, Yershov states in the first sentence of the Abstract, "We present a further development in the technology of <u>sequencing</u> by hybridization to oligonucleotide microchips (SHOM)." (Emphasis added.) In the final paragraph of the first column on page 4913, Yershov states, "Here we report an advance in the development of SHOM technology and SHOM application for sequence analysis and diagnostics." (Emphasis added.) Yershov begins a section on page 4916 entitled, "Identification of Base Changes in DNA, by stating, "The reliability of <u>sequence</u> analysis by SHOM . . . ." (Emphasis added.) Similarly, Yershov begins the "Conclusions" section at page 4917, by stating, "The results presented here show that oligonucleotide microchips can be effectively used for <u>sequence</u> analysis . . . ." (Emphasis added.) Accordingly, the intent and purpose of the Yershov publication was to improve the methodology of <u>sequencing</u> by hybridization to oligonucleotide microchips.

Likewise, the first sentence of the '134 patent Abstract states, "This invention is directed to methods for determining a nucleotide <u>sequence</u> of a nucleic acid . . . ." (Emphasis added.) The first sentence of the Summary of the Invention also emphasizes sequencing, as follows.

> The present invention overcomes the problems and disadvantages associated with current strategies and design and provides a new method for rapidly and accurately <u>determining the nucleotide sequence</u> of a nucleic acid . . . .

'134 patent, col. 3, lines 25-28 (emphasis added). Similarly, the second sentence of the Summary of the Invention states, "As broadly described herein, this invention is directed to a rapid, accurate, and reproducible method of <u>sequencing a nucleic acid</u> . . . ." Col. 3, lines 32-33

(emphasis added). The third paragraph of the Summary of the Invention begins, "As broadly described herein, another object of this invention is the integration of molecular biology techniques to the method of positional sequencing . . . ." Col. 3, lines 43-45. Examples 4, 6 and 8 as well as Figures 4, 7 and 8 teach primer extension of hybridized nucleic acids. Applicants submit that while the Examiner has stated that the '134 patent at column 5, line 56 to column 6, line alleged teaches certain aspects of the invention, the Examiner has failed to note that the paragraph containing the cited text begins, "One embodiment of the present invention is a method for determining the nucleotide sequence." Col. 5, lines 47-48. Similarly, the Examiner has pointed to the '134 patent at Col. 8, lines 1-10 but this text clearly recites that step (d) involves "determining the nucleic sequence of the target. . ." Col. 8, line 9. Accordingly, the '134 patent discloses and teaches methods of sequencing a nucleic acid.

Applicants further submit that the present methods avoid many of the problems associated with sequencing. For example, Cantor complains about prior art sequencing procedures as follows:

> Despite their advantages, these procedures are cumbersome and impractical when one wishes to obtain megabases of sequence information. Further, these procedures are, for all practical purposes, limited to sequencing DNA. Although variations have developed, it is still not possible using either process to obtain sequence information directly from any other form of nucleic acid.

'134 Patent, col. 1, line 63 to col. 2, line 2.

> In spite of an overall optimistic outlook, there are still a number of potentially severe drawbacks to actual implementation of sequencing by hybridization. First and foremost among these is that 4^n rapidly becomes quite a large number if chemical synthesis of all of the oligonucleotide probes is actually contemplated. Various schemes of automating this synthesis and compressing the products into a small scale array, a sequencing chip, have been proposed.
>
> A second drawback is the poor level of discrimination between a correctly hybridized, perfectly matched duplexes, and an end mismatch.

'134 Patent, col. 1, lines 37–47.

> A third drawback is that detection is monochromatic. Separate sequential positive and negative controls must be run to discriminate between a

> correct hybridization match, a mis-match, and background.
>
> A fourth drawback is that ambiguities develop in reading sequences longer than a few hundred base pairs on account of sequence recurrences. For example, if a sequence the same length of the probe recurs three times in the target, the sequence position cannot be uniquely determined. The locations of these sequence ambiguities are called branch points.
>
> A fifth drawback is the effect of secondary structures in the target nucleic acid. This could lead to blocks of sequences that are unreadable if the secondary structure is more stable than occurs on the complementary strand.
>
> A final drawback is the possibility that certain probes will have anomalous behavior and for one reason or another, be recalcitrant to hybridization under whatever standard sets of conditions that are ultimately used. A simple example of this is the difficulty in finding matching conditions for probes rich in G/C content. A more complex example could be sequences with a high propensity to form triple helices. The only way to rigorously explore these possibilities is to carry out extensive hybridization studies with all possible oligonucleotides of length n, under the particular format and conditions chosen. This is clearly impractical if many sets of conditions are involved.

'134 Patent, col. 2, line 64 to col. 3, line 22. Hence, many problems and drawbacks are inherently present in sequencing procedures that can confound and delay resolution of a DNA or RNA sequence.

Use of the present methods avoids all these problems and drawbacks. By comparing reference and target hybridization patterns, after hybridization to identical polynucleotide probe arrays, differences can quickly be identified. No need exists for labor-intensive resolution of branch-point and repetitive sequence problems. If a difference is noted in target hybridization pattern compared to the reference hybridization pattern, the two nucleic acids are different. Hence, large numbers of samples can rapidly and accurately be screened to check for any type of difference (e.g., mis-match, deletion, substitution or other mutation). *See* Specification at page 15, lines 1-9; page 15, lines 19-29. Use of a complete set of n-mers, insures that any difference will be identified. Elimination of the sequencing step streamlines and simplifies the analysis, thereby providing a rapid, cost-effective method for determining whether two or more target

polynucleotides are identical and for identifying the presence of a mutation in a target polynucleotide.

Additionally, sequencing by hybridization cannot unambiguously identify a base and the likelihood is high that there will be some percentage of error due to hybridizing the unknown target to the array under one set of conditions. This issue will not present a problem in the current invention because the goal is merely to provide comparisons of reproducible hybridization patterns and it is not relevant if there is a mismatch between probe and target, for example, when a different set of hybridization conditions is used.

The Examiner has alleged that one cannot show nonobviousness by attacking references individually. Official Action at 5 (Apr. 4, 2001). However, as indicated above, there must be some suggestion or motivation, either in the cited reference or in the knowledge generally available to one of ordinary skill in the art, to modify a reference or to combine reference teachings so as to arrive at the claimed invention. M.P.E.P. § 2142. Here, no motivation exists in the two references to combine them. And further, as discussed above, even if they were combined, they teach away from the claimed invention. Moreover, even when the '134 patent and the Yershov reference are combined, this combination does not teach the methods of the invention. Hence, this combination of references cannot render the claimed invention obvious. *See in re Vaeck*, 20 USPQ2d at 1442, 1444.

Applicants submit that because the combination of the '134 patent and Yershov does not teach the subject matter of independent claims 1 or 12, all of claims 1-18 are non-obvious in view of this combination of references. Therefore, Applicants respectfully request withdrawal of the rejection of claims 1-18 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,631,134 to Cantor et al. (the '134 patent) in view of Yershov et al., 93 Proc. Natl. Acad. Sci., USA 4913-18 (1996).

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney 612-373-6961 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 C.F.R. 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 5 day of July, 2001.

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